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Oxidative stress in rat striatum after pilocarpine-induced seizures is diminished by alpha-tocopherol

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ABSTRACT

Alpha-tocopherol has numerous nonenzymatic actions and is a powerful liposoluble antioxidant. The objective of the present study was to evaluate the neuroprotective effects of alpha-tocopherol in rats against oxidative stress caused by pilocarpine-induced seizures. Wistar rats were intraperitoneally treated with 0.9% saline (control group), alpha-tocopherol (200 mg/kg, alpha-tocopherol group), pilocarpine (400 mg/kg, pilocarpine group), or the combination of alpha-tocopherol (200 mg/kg) and pilocarpine (400 mg/kg, i.p.; alpha-tocopherol plus pilocarpine group). After the treatments, all groups were observed for 24 h. The superoxide dismutase (Mn-SOD) and catalase activities, lipid peroxidation and nitrite concentrations were measured using spectrophotometrically methods. To clarify the mechanism of alpha-tocopherol on oxidative stress in pilocarpine model, Western blot analysis of Mn-SOD and catalase in rat striatum were performed. In the pilocarpine group, rats showed a significant increase in lipid peroxidation and nitrite levels. However, there were no alterations on Mn-SOD activity. On the other hand, the catalase activity augmented in pilocarpine group. In the alpha-tocopherol and pilocarpine co-administered rats, antioxidant treatment significantly reduced the lipid peroxidation level and nitrite content and increased the Mn-SOD and catalase activities in rat striatum after seizures. Pilocarpine, alpha-tocopherol plus pilocarpine and alpha-tocopherol groups did not affect of the Mn-SOD and catalase mRNA or protein levels. Our findings strongly support the hypothesis that oxidative stress occurs in striatum during pilocarpine-induced seizures, indicating that brain damage induced by the oxidative process plays a crucial role in seizures pathogenic consequences, which implies that strong protective effect could be achieved using alpha-tocopherol.

1. Introduction

The animal model induced by pilocarpine is very useful to study the development and neuropathology of temporal lobe epilepsy (Treiman, 1995; Turski et al., 1983). Neurochemical as well as enzymatic activities studies suggest that excitotoxic stimulation in status epilepticus induces oxidative stress (Barros et al., 2007; Frantseva et al., 2000; Freitas et al., 2005). Through the regulation of reactive oxygen species by production and maintenance of oxidative phosphorylation, the alpha-tocopherol acts as antioxidant and protects cell membrane (Ayyildiz et al., 2006; Kotegawa et al., 1993; Levy et al., 1982; Tomé et al., 2010a).

In recent years, a great deal of attention has been given to antioxidants consumption and their roles in reducing rates of chronic diseases such as epilepsy, inflammation and cancer (Carr and Frei, 1999; Ferreira et al., 2008; Simon et al., 2001; Tomé et al., 2010a). It is suggested that protective effects of compounds is partly due to antioxidant which inhibit lipid peroxidation and nitrite formation (Steinmetz and Potter, 1991; Xavier et al., 2007). Reactive oxygen species have been implicated in the development of seizures induced by pilocarpine (Santos et al., 2008; Tomé et al., 2010b). The mechanism underlying seizures-induced oxidative stress is poorly understood though several interpretations have been proposed (Liang et al., 2007; Tomé et al., 2010b). Recently, several studies have examined the role of oxidative stress on pilocarpine-induced seizures which was thought possibly via the formation of free radicals (Freitas, 2009; Freitas et al., 2004; Tomé et al., 2010b).

Reactive oxygen species are generated during oxidative stress, being as a possible mechanism to pilocarpine-induced seizures. The brain processes large amounts of O_2 in relatively small mass and has a

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high content of substrates available for oxidation in conjunction with low antioxidant activities, making it extremely susceptible to oxidative damage (Dal-Pizzol et al., 2000; McCord, 1989). In addition, certain regions of Central Nervous System, such as striatum, are particularly sensitive to oxidative stress because of their low endogenous levels of alpha-tocopherol (Bergamini et al., 2004; Gottlieb et al., 2006), and under pro-oxidative conditions such as those in seizures, the low antioxidant defenses capacity can predispose the striatum to oxidative stress.

A variety of animal seizure models aid in documenting the effects of alpha-tocopherol and specifying its action (Ayyildiz et al., 2006; Kryzhanovskii et al., 1980). Preliminary injection of alpha-tocopherol in rats abolished the effect of lipid peroxidation activation and decreased the number of seizures (Kryzhanovskii et al., 1980; Tomé et al., 2010a). Treatment with alpha-tocopherol prevented this dropping, which supports that it has neuroprotective effect (Kovacs et al., 2005; Tomé et al., 2010b). The aim of present study was to examine the effects of alpha-tocopherol on oxidative stress in rat striatum after 24 h of phase acute of pilocarpine-induced seizures.

2. Material and methods

2.1. Animals and reagents

Adult male Wistar rats (250–280 g; 2 months old) were maintained in a temperature controlled room (26 ± 1 °C) with a 12-h light/dark cycle and food and water *ad libitum* (Nutrilabor, Campinas, Brazil). All experiments were performed according to the guide for the care and use of laboratory the US Department of Health and Human Services, Washington, DC (1985). The dosages of pilocarpine hydrochloride and alpha-tocopherol (Sigma, Chemical USA) are expressed at milligrams per kilogram of body weight, and were administered in a volume of 10 ml/kg injected intraperitoneally (i.p.).

2.2. Experimental procedures

A total of 96 rats were intraperitoneally treated with either 200 mg/kg alpha-tocopherol or 0.9% saline. Thirty minutes after the treatments twenty-four rats from each above group were randomized to pilocarpine hybrochloride administration (400 mg/kg, pilocarpine group). Thus, there are 4 groups of rats in this set of experiments: group 1, alpha-tocopherol and pilocarpine co-administration ($n=24$); group 2, pilocarpine plus saline treatment ($n=24$); group 3, alpha-tocopherol alone administration ($n=24$); and group 4, saline treatment (negative control, $n=24$). After the treatments, the animals were recorded in 30 cm × 30 cm chambers with: latency to first seizure (any one of the behavioral indices typically observed after pilocarpine administration: wild running, clonuses, tonus, clonic-tonic seizures), number of animals that died after pilocarpine administration. Previously, a work showed that convulsions and deaths occurred within 1 and 24 h respectively post pilocarpine injection, so we decided to record the phenotypes of the animals for 24 h after pilocarpine administration.

At the end of observations, the survivors were killed by decapitation and their brains were dissected out on ice to remove striatum for histophatological and Western blot analyses and determinations of lipid peroxidation level, nitrite content, superoxide dismutase (Mn-SOD) and catalase activities.

The pilocarpine administration rat group was constituted by those presented seizures, SE for over 30 min and non-phenotype survivors. Doses of pilocarpine (400 mg/kg) and alpha-tocopherol (200 mg/kg) were determined by previous study in our lab (Barros et al., 2007; Tomé et al., 2010a; Xavier et al., 2007).

2.3. Determinations of lipid peroxidation level in rat striatum pretreated with alpha-tocopherol after 24 h of phase acute of pilocarpine-induced seizures

For all experimental procedures, 10% (w/v) homogenates of the area of brain investigated were prepared for all groups. Lipid peroxidation levels in the alpha-tocopherol plus pilocarpine group ($n=6$), pilocarpine group ($n=6$), alpha-tocopherol group ($n=6$) and control animal ($n=9$) were analyzed by measuring the thiobarbituric-acid-reacting substances in homogenates (Draper and Hadley, 1990), and expressed as nmol of malondialdehyde (MDA)/g wet tissue.

2.4. Determinations of nitrite content in rat striatum pretreated with alpha-tocopherol after 24 h of phase acute of pilocarpine-induced seizures

To determine nitrite contents of control group ($n=9$), alpha-tocopherol plus pilocarpine group ($n=6$), pilocarpine group ($n=6$) and alpha-tocopherol group ($n=6$), the 10% (w/v), homogenates were centrifuged (800×g, 10 min). The supernatants were collected, and nitric oxide production was determined based on the Griess reaction (Green et al., 1981). The results above were expressed as nM.

2.5. Determinations of superoxide dismutase activity in rat striatum pretreated with alpha-tocopherol after 24 h of phase acute of pilocarpine-induced seizures

The striatum was ultrasonically homogenized in 1 ml 0.05 M sodium phosphate buffer, pH = 7.0. Protein concentration was measured (Lowry et al., 1951). The 10% homogenates were centrifuged (800×g, 20 min) and supernatants were used to assay superoxide dismutase (Mn-SOD) and catalase. Mn-SOD activity in the alpha-tocopherol plus pilocarpine group ($n=6$), pilocarpine group ($n=6$) and alpha-tocopherol group ($n=6$) and control animals ($n=9$) was assayed by using xanthine and xanthine oxidase to generate superoxide radicals (Flohe and Otting, 1984), and the results were expressed as U/mg of protein.

2.6. Determinations of catalase activity in striatum of adult rats pretreated with alpha-tocopherol after 24 h of phase acute of pilocarpine-induced seizures

Catalase activity was measured in the alpha-tocopherol plus pilocarpine group ($n=6$), pilocarpine group ($n=6$) and alpha-tocopherol group ($n=6$) and control ($n=9$) groups by the method that uses H_2O_2 to generate H_2O and O_2 (Chance and Maehly, 1955). Results were expressed as mmol/min/mg of protein.

2.7. Determinations of histopathological changes in rat striatum pretreated with alpha-tocopherol after 24 h of phase acute of pilocarpine-induced seizures

All groups were observed during 24 h for behavioral changes and convulsive state. After that, animals were sacrificed by decapitation 24 h and brains were dissected out and fixed in formalin 10% (Marinho et al., 1998; Szyndler et al., 2005). After an initial coronal section at the level of the optic nerve, 3–5 μm thick sections were prepared and stained with Hematoxylin & Eosin (HE) for light microscopy studies (100×). The degree of striatal damage severity was defined by a scale ranging from 0 (none) to 100 (total) by light microscopy and previously defined to be reliable for morphological analysis (Paxinos and Watson, 1986). Brain damage presence was confirmed if striatum showed at least 50% involvement.

2.8. Western blot analysis

For immunoblotting, striatum homogenates were mixed with protein loading buffer (roti-Load 1, Carl Roth GmbH, Karlsruhe, Germany) according to manufacturer's procedure and placed in a heating bath (95 °C) for 5 min. Proteins were separated using SDS-PAGE (gradient gels from 5% to 25%). The protein amount loaded per lane was 10 Ag. After separation, the proteins were stained with Coomassie Brilliant Blue or transferred to nitrocellulose paper and unspecific protein binding sites were blocked with blocking buffer (Chemicon International, Hofheim, Germany). The blots were incubated overnight with the primary antibodies against (1) catalase (polyclonal, UBI, Lake Placid, NY, USA, 1:1.500) and (2) Mn-SOD (polyclonal, Assayama, Japan, 1:800), followed by incubation with horseradish peroxidase-conjugated secondary antibody (goat antirabbit IgG + peroxidase, Boehringer Mannheim GmbH, Germany, 1:1.000). Immunoreactivity was visualized using the ECL detection system (Amersham Pharmacia Biotech, Buckinghamshire, UK).

2.9. Statistical analysis

Results of latency to first seizure, histopathological abnormalities and neurochemical alterations were compared by one-way analysis of variance (ANOVA) followed by *t*-Student-Newman-Keuls test ($p<0.05$) (Graphpad program Intuitive, Software for Science, San Diego, CA). The number of animals that seized and the number that survived were calculated as percentages (seizures percentage and survival percentage, respectively), and compared with a nonparametric test (χ^2).

3. Results

3.1. Behavioral alterations after pretreatment with alpha-tocopherol after 24 h of phase acute of pilocarpine-induced seizures

Pilocarpine induced the first seizure at 34.65 ± 1.63 min. All animals studied showed generalized tonic-clonic convulsions with status epilepticus, and 40% survived the seizures. All animals pretreated with alpha-tocopherol were observed for 24 h before pilocarpine injection and they manifested alterations in behavior, such as peripheral cholinergic signs (100%), tremors (50%), staring spells, facial automatisms, wet dog shakes, rearing and motor seizures (45%) developed progressively within 1–2 h into a long-lasting status epilepticus (45%). Table 1 shows that alpha-tocopherol administered (200 mg/kg; alpha-tocopherol plus pilocarpine) before pilocarpine reduced by 55% the percentage of animals that seized, increased latency (261%) to the first seizure (125.14 ± 1.95 min) and increased (20%) the survival ($P<0.0001$) when compared to the pilocarpine group. None of the control animals (saline or alpha-tocopherol) showed seizures (Table 1).

Table 1
Effect of pretreatment with alpha-tocopherol after pilocarpine-induced seizures.

Groups	Latency to first seizures (min)	Percentage seizures	Percentage survival	Number of animals/group
Control	00	00	00	00
Pilocarpine	34.65 ± 1.63	100	40	24
Alpha-tocopherol plus pilocarpine	125.14 ± 1.95[a]	45[b]	60[b]	24
Alpha-tocopherol	00	00	100[b,c]	24

Male rats (250–280 g, 2 months old) were intraperitoneally treated with a single dose of pilocarpine (400 mg/kg, pilocarpine group) and alpha-tocopherol group with alpha-tocopherol (200 mg/kg, alpha-tocopherol group). The alpha-tocopherol plus pilocarpine group was treated with alpha-tocopherol (200 mg/kg) for 30 min before pilocarpine injection (400 mg/kg, alpha-tocopherol plus pilocarpine group). Results for latency to first seizure are expressed as mean ± S.E.M of the number of experiments shown in the table. Results for percentage seizures and percentage survival are expressed as percentages of the number of animals from each experimental group.

[a] $P<0.05$ compared with pilocarpine group (ANOVA and *t*-Student–Newman–Keuls test).

[b] $P<0.05$ as compared with P400 group (χ^2-test).

[c] $P<0.05$ compared with alpha-tocopherol plus pilocarpine group (χ^2-test).

3.2. Lipid peroxidation level and nitrite content in rat striatum pretreated with alpha-tocopherol after 24 h of phase acute of pilocarpine-induced seizures

Effects of alpha-tocopherol in lipid peroxidation and nitrite concentrations during seizures induced by pilocarpine are presented in Fig. 1. Lipid peroxidation was markedly increased in pilocarpine group in comparison with the saline group. During acute phase of seizures induced by pilocarpine a significant increase (47%) in thiobarbituric-acid-reacting substances was observed ($P<0.0001$). Seizures induced by pilocarpine produced a significant increase in striatal nitrite content (49%, $P<0.0001$, Fig. 1). Alpha-tocopherol pretreated rats showed decrease in lipid peroxidation level (77%) and nitrite content (44%) when to compared with the pilocarpine group ($P<0.0001$, Fig. 1). Additionally, the pretreatment with alpha-tocopherol 30 min before administration of pilocarpine also reduced lipid peroxidation level (66%) and nitrite content (17%) when compared to the control group ($P<0.001$, Fig. 1). On the other hand, animals of the controls animals (saline or alpha-tocopherol) showed no alterations in lipid peroxidation level and nitrite content (Fig. 1).

3.3. Superoxide dismustase and catalase activities in rat striatum pretreated with alpha-tocopherol after 24 h of phase acute of pilocarpine-induced seizures

Mn-SOD activity in rat striatum during acute phase of seizures was not markedly altered in pilocarpine group in comparison with control saline group though catalase activity during acute phase of seizures has markedly increased in pilocarpine group (39%; $P<0.0001$). Correspondingly, there was a significant increase Mn-SOD (7 and 8%) and catalase (20 and 56%) activities of rats pretreated with alpha-tocopherol in comparison to the pilocarpine and control groups, respectively ($P<0.0001$) (Fig. 1). On the other hand, no enzymatic alterations occurred in alpha-tocopherol group (Fig. 1).

3.4. Histopathological changes in rat striatum pretreated with alpha-tocopherol after 24 h of phase acute of pilocarpine-induced seizures

Brain tissue examinations of the control and alpha-tocopherol groups did not reveal striatal histological changes. On the other hand, pilocarpine group presented neuronal loss, gliosis, and typical vacuolar degeneration in striatum region. Histopathological damages were observed in four (80%) rats of pilocarpine group, and none of the control animals, alpha-tocopherol plus pilocarpine and alpha-tocopherol groups presented striatal morphological changes (Fig. 2).

3.5. Results of Western blot analysis of Mn-SOD and catalase in rat striatum pretreated with alpha-tocopherol after 24 h of phase acute of pilocarpine-induced seizures

The results obtained by Mn-SOD and catalase activities in rat striatum pretreated with alpha-tocopherol after 24 h of phase acute of pilocarpine-induced seizures measurements could be further supported by Western blot analysis. To clarify the mechanism of alpha-tocopherol on oxidative stress for the development of anticonvulsant effects in pilocarpine model, the following results of Western blot analysis of Mn-SOD and catalase in rat striatum homogenates were obtained after 24 h of phase acute of pilocarpine model. After 24 h of acute phase of seizures induced by pilocarpine (400 mg/kg) the total Mn-SOD and catalase activities no were changed in comparison with saline controls. Likewise, alpha-tocopherol plus pilocarpine and alpha-tocopherol groups did not

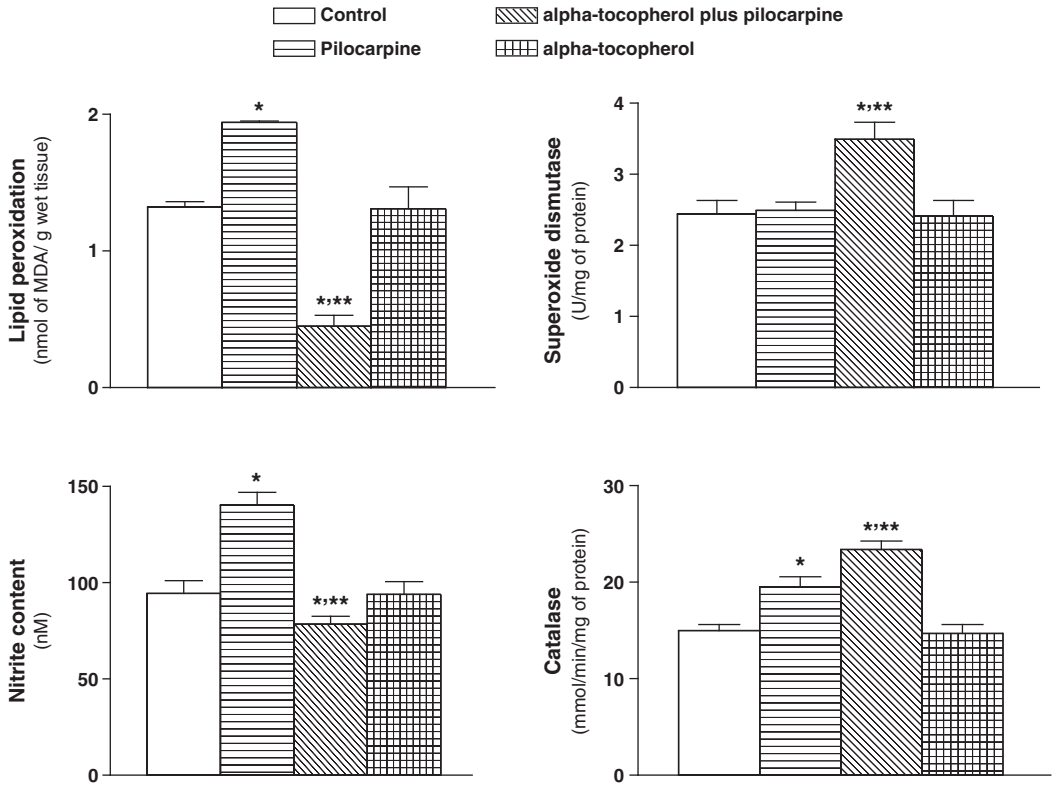


Fig. 1. Effects of alpha-tocopherol on status of lipid peroxidation level, nitrite content, and antioxidant enzymes activities in rat striatum after 24 h of phase acute of pilocarpine-induced seizures. Male rats (250–280 g, 2 months-old) were intraperitoneally treated with a single dose of pilocarpine (400 mg/kg, $n=6$, pilocarpine group), alpha-tocopherol group with alpha-tocopherol (200 mg/kg, $n=6$, alpha-tocopherol group) and the control animals with 0.9% saline ($n=9$, Control). The alpha-tocopherol plus pilocarpine group was treated with alpha-tocopherol (200 mg/kg) for 30 min prior to pilocarpine injection (400 mg/kg, $n=6$, alpha-tocopherol plus pilocarpine group). Results are expressed as means ± S.E.M. for the number of animals shown inside in parenthesis. Differences in experimental groups were determined by two-tailed Analysis of Variance (ANOVA). $^{*}P<0.05$ compared to the control group (ANOVA and *t*-Student–Neuman–Keuls test); $^{**}P<0.05$ compared to the pilocarpine group (ANOVA and *t*-Student–Neuman–Keuls test).

affect of the Mn-SOD and catalase mRNA or protein levels, as tested by immunoblot analyses of striatal homogenates of rat pretreated with alpha-tocopherol after 24 h (Fig. 3).

4. Discussion

The main result of this work is the fact that the antioxidant drug studied produced dramatic decrease in lipid peroxidation and nitrite content in rat brain striatum elevated as a result of pilocarpine-induced seizures. It is also supported by the report that the lipid peroxidation and nitrite content in the hippocampus is suppressed by an endogenous antioxidant alpha-tocopherol (Tomé et al., 2010a). Recently published data demonstrated that alpha-tocopherol, produced the anticonvulsive action against pilocarpine-induced seizures, increasing latency to first seizures (Tomé et al., 2010b).

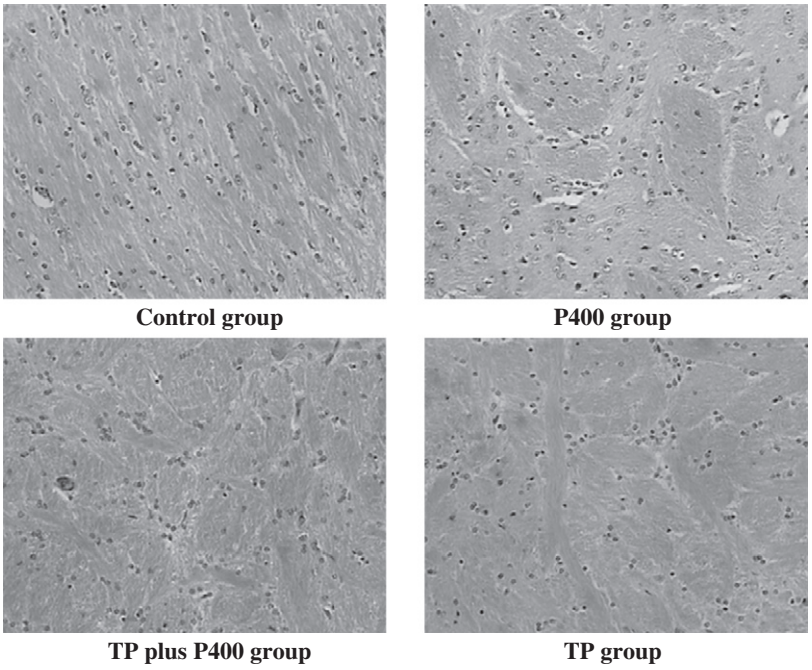
The mechanism of this phenomenon is still unclear. The current study also sought to determine the anticonvulsant and neuroprotective action of antioxidant alpha-tocopherol. Alpha-tocopherol has been reported to be effective in reducing the consequences of epilepsy model induced by pilocarpine (Tomé et al., 2010a). In our experiments, alpha-tocopherol prevented the tonic phase of pilocarpine-induced seizures and significantly diminished the clonic phase of convulsions, as well as completely abolished the pilocarpine-induced increases in nitrite and thiobarbituric-acid-reacting substances formation. Our findings indicated that alpha-tocopherol was highly effective in the prevention of pilocarpine-induced seizures, and these data are predictive of its possible clinical applications. These data may support our concept that alpha-tocopherol neuroprotection is correlated with its ability to inhibit not only excessive reactive oxygen species formation but also nitrite generation directly by inhibiting one of several events that contribute to nitrite production.

The molecular observations of epilepsy include the temporal correlation between free radical generation and the development of seizures in some pathological conditions, and the protective efficacy of antioxidant treatments against some types of seizures. Alpha-tocopherol, one of the effective antioxidant, not only has antioxidant functions, but also has functions in pro-oxidant, cell signaling and gene regulation (Godlevskii et al., 2004; Sudha et al., 2001; Tucker and Townsend, 2005). Previous studies indicated that alpha-tocopherol has anticonvulsant activity in several animal models (Jerrett et al., 1973; Tucker and Townsend, 2005). In this study, we demonstrated a role of alpha-tocopherol against oxidative stress in rat striatum generated after 24 h of phase acute of pilocarpine-induced seizures.

It has long been recognized that the striatum is one of the major structures which are preferentially involved in epilepsy model induced by pilocarpine (Freitas et al., 2004). More recently, studies of clonic-tonic seizures by method revealed a remarkable increase of reactive oxygen species formation in the hippocampus, frontal cortex and striatum as well as in the focus itself (Freitas et al., 2005; Militão et al., 2010).

However, the possible significance of striatal involvement in epileptic seizures induced by pilocarpine remains unclear as to whether it exerts a suppressive or facilitatory effect on seizure development. This uncertainty is reflected by conflicting reports of a seizure-suppressive effect of caudate stimulation on the one hand, and the generation of myocionus or generalized convulsive seizure by intrastriatal injection of direct or indirect neuroexcitants on the other. Also, there are opposing opinions as to the function of the *substantia nigra* and the *globus pallidus*, which are the output stations of the striatal influences.

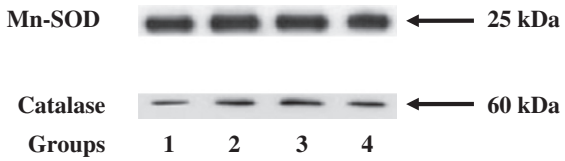
Generation of reactive oxygen species is currently viewed as one of the process through which epileptic activity exert their deleterious



Groups	Rats with lesion (%)	Severity of lesion (%)	Number of animals with lesion per group
Control	00	00	00
Pilocarpine	80	55.39 ± 0.52	04
alpha-tocopherol plus pilocarpine	00	00	**0**
alpha-tocopherol	00	00	**0**

Fig. 2. Histopathological alterations in striatum of rats pretreated with alpha-tocopherol after 24 h of phase acute of pilocarpine-induced seizures. Severity of lesion was expressed as a mean ± S.E.M. of scores of damage based in a scale from zero (none) to 100 (total) percentage of striatum involvement. Brain damage was considered positive if there was at least 50% striatal involvement. Hematoxylin and Eosin staining (HE). Magnification, 100×. Figures shown is from one representative experiment of $n = 5$ per group.

effects on brain (Rauca et al., 2004). These reactive oxygen species in the absence of an efficient defense mechanism cause lipid peroxidation (Castagne et al., 1999). We showed growing lipid peroxidation in rat striatum after 24 h of acute phase of seizures. The increase of lipid peroxidation was reflected by the rise of thiobarbituric-acid-reacting substances level which may be related to its intermediate free radicals formed during seizures induced by pilocarpine. The striatum is more vulnerable to injury by lipid peroxidation products than other rat hippocampus (Barros et al., 2007; Tomé et al., 2010a). Moreover, lipid peroxidation is an index of irreversible neuronal damage of cell membrane phospholipid and it has been suggested as a possible mechanism of epilepsy model activity during acute phase of seizures. Our findings show an increase in lipid peroxidation and nitrite contents in this phase of seizures.



Legends: 1 – Control group**;**
2 – Pilocarpine group**;**
3 – alpha-tocopherol plus pilocarpine group**;**
4 – alpha-tocopherol group**.**

Fig. 3. Western blot analysis of Mn-SOD and catalase in rat striatum after 24 h of acute phase of seizures induced by pilocarpine. Male rats (250–280 g, 2 months-old) were intraperitoneally treated with a single dose of pilocarpine (400 mg/kg, $n = 6$, pilocarpine group), alpha-tocopherol group with alpha-tocopherol (200 mg/kg, $n = 6$, alpha-tocopherol group) and the control animals with 0.9% saline ($n = 9$, Control). The alpha-tocopherol plus pilocarpine group was treated with alpha-tocopherol (200 mg/kg) for 30 min prior to pilocarpine injection (400 mg/kg, $n = 6$, alpha-tocopherol plus pilocarpine group). Animals were observed for 24 h and then killed. The protein amount per lane was 10 Ag. Shown is one representative experiment of $n = 6$. Legends: 1 — Control group; 2 — Pilocarpine group; 3 — alpha-tocopherol plus pilocarpine group; 4 — alpha-tocopherol group.

Literature has shown that pilocarpine-induced seizures led to changes in nitric oxide metabolism, and increased the production of its metabolites (nitrite and nitrate). The increased metabolites may interact with glutamatergic receptors to produce part of its stimulatory action on the central nervous system (Maczurek et al., 2008; Michiels et al., 1994). The reduction in nitrite content after pretreatment with alpha-tocopherol is most readily explained as consequence of radical formation inhibiting, scavenger of reactive oxygen species and lipid peroxidation products (Tejada et al., 2006).

Histopathological studies of animals pretreated with alpha-tocopherol showed a decrease of 80% in the number of animals presenting striatal damage after 24 of acute phase of seizures. These findings support the theory of the oxidative stress involvement in the start of seizures by the increase of free radical production and suggest a neuroprotective activity of alpha-tocopherol by the removal of free radicals produced during pilocarpine-induced seizures. Moreover, the results suggest that oxidative stress mediated by pilocarpine exerts its pathologic effects and also that the neuroprotective and anticonvulsive role of alpha-tocopherol can be

mediated by a reduction in lipid peroxidation levels and nitrite content. Possibly, this reduction is due to the modulatory activity of alpha-tocopherol in the antioxidant enzymes (Mn-SOD and catalase) in rat striatum.

Mn-SOD activity does not protect rat striatum against damage neuronal caused by seizures induced by pilocarpine since Mn-SOD activity no changes was observed during acute phase of seizures. On the other hand, the catalase activity augmented in those animals presenting seizures, indicating that H_2O_2 generated during superoxide dismutation would not be sufficiently removed by catalase during acute phase of seizures. The increase in antioxidant enzymes activities induced by alpha-tocopherol might be explained as a necessary consequence of scavenging of $O_2^{\cdot}$ produced by dismutation.

Free radical formation elevations are frequently accompanied by an immediate compensatory increase in the activities of the free radical scavenging enzymes (Salo et al., 1988). Previous studies have showed an increased in catalase activity in the striatum during a 24 h period of acute phase of seizures (Barros et al., 2007; Salo et al., 1988). The capability of alpha-tocopherol to increase antioxidant enzymes activities and decrease free radical formation will finally lead to a significant decrease in the susceptibility to seizures induced by pilocarpine.

Our findings showed that pilocarpine-induced seizures can produce alterations in catalase activity in rat hippocampus, thereby protecting the brain from neuronal damage induced by lipid peroxidation products. It is unlikely that the unaltered glutathione peroxidase activity is related to the mechanisms involved in maintenance of seizures. Our results corroborate with another study showing augmented hippocampal superoxide dismutase and activity after 24 h of acute phase of seizures (Barros et al., 2007; Tomé et al., 2010a), suggesting that superoxide dismutase and catalase activities only changes during the seizures (Tomé et al., 2010b). When studying this acute phase of epilepsy model, we found increased catalase activity in the hippocampus, indicating that this enzyme, in association with reduced glutathione, provides neuroprotection against the oxidative stress. These data suggest that hippocampus does not use glutathione peroxidase as the major free radical scavenging system (Júnior et al., 2009; Michiels et al., 1994; Militão et al., 2010).

In our studies, the total Mn-SOD and catalase activities no were changed during acute phase of seizures induced by pilocarpine. The data of Western blot analysis no demonstrated evidence for the upregulation of antioxidant enzymes (Mn-SOD and catalase) after pilocarpine-induced seizures after 24 h of phase acute. In addition, the data obtained with Western blot analysis confirmed our hypothesis that occurred only an increase in the enzymatic activities studied, since there was no change in protein contents of Mn-SOD and catalase in rat striatum after 24 h of phase acute of pilocarpine-induced seizures.

We clearly showed that alpha-tocopherol decreased the frequency of pilocarpine-induced seizures and increased the survival rate. In our knowledge, these effects of alpha-tocopherol on oxidative stress observed during acute phases of pilocarpine-induced seizures have not been reported before. Thus, these findings might have important implications for understanding the mechanism of epilepsy to promote new advances in the development of selective and targeted antiepileptic drugs. Alpha-tocopherol protected the striatum against neuronal damages regularly observed during seizures. Further investigations of the effects of alpha-tocopherol against necrosis, apoptosis and/or autophagy observed during the acute phase of this epilepsy model are in progress to confirm its neuroprotective effects.

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